FOREST INSECT AND DISEASE CONTROL
STATE AND PRIVATE FORESTRY

REGION 4 FOREST SERVICE
U.S. DEPARTMENT OF AGRICULTURE
OGDEN, UTAH
JULY 1976

A PRELIMINARY ANATOMICAL STUDY OF THE OUTER TISSUES OF LODGEPOLE PINE BOLES FOLLOWING TREATMENT WITH FUEL OIL FORMULATED INSECTICIDES

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by

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INTRODUCTION

On June 16, 1975, a protective spray project was initiated by the U.S. Forest Service in Buffalo Campground, Targhee National Forest, on lodgepole pine, Pinus contorta Dougl., to protect trees from the mountain pine beetle Dendroctonus ponderosae Hopk. 1/ The project entailed spraying tree boles with the insecticides, Dursban, Lindane, and Sevin, diluted to 2% concentration in #2 fuel oil on 200 trees per insecticide throughout the campground. Tree boles were completely sprayed from the ground level up to 25 feet in all cases.

During the latter part of September 1975 an apparent phytotoxic reaction was noted on treated trees in the form of "resin-like" exudation beginning at approximately the 25-foot level (Figures 1 and 2). Photosynthate products and/or resin exudate at this level was noted on trees sprayed with each mixture indicating the phytotoxic reaction was related to the base medium #2 fuel oil.

The following is a preliminary study to determine the cause of the observed phytotoxic reaction through an anatomical study of outer tree tissues.

METHODS

Lodgepole pine samples were collected at the end of the 1975 growing season, or approximately three months after spraying had been completed. Five two-centimeter-square sections of bark and recent secondary xylem were removed from three lindane treated trees and two adjacent non-treated trees. Non-treated trees had not been exposed to the spray materials and were in the same diameter class as treated trees. Selected treated trees exhibited high exudation levels. Beginning at the 25-foot level, sections from each tree were removed at 1-foot intervals moving vertically down the tree until 5 blocks had been collected. Upon collection, the sections were labeled, placed in a formaldehyde-acetic acid-alocohol fixative, and later dehydrated through the following series:

1/ Klein, William H. 1975. Working Plan. Pilot test of three insecticides for preventing attacks of the mountain pine beetle in lodgepole pine. U.S. Forest Service, Ogden, Utah. 2 Figs. 3 tables. 7 p.

Grade No.	95% Ethanol	Absolute Ethanol	Tertiary Butyl Alcohol	H_2O
1	50	-	10	40
2	50	-	20	30
3	50	-	35	15
4	50	-	50	-
5	25	-	75	-
6	-	25	75	-
7	-	-	100	-

Following dehydration, blocks were placed in a paraffin oil-tertiary butyl alcohol 1/1 mixture for 10 hours and then placed with the cited mixture on a solid paraplast block in a glass vial. The vial was then placed in an oven at 45° C which allowed the wood sections to melt down through the paraplast and be infiltrated. The blocks were then removed and embedded in pure paraplast for sectioning. Sectioning was done with a rotary microtome with only radial and transverse sections being prepared for study. Safranin O and fast green at .5% concentrations were used to stain the prepared sections.

OBSERVATIONS

Since each tissue performs a specific role in the function of the tree, and responds in various ways to foreign substances, each will be discussed separately. In general, bark tissues have been adversely affected with most modes of synthate transport appearing to be involved. In numerous sections, no tissues could be distinguished in the inner bark. The effect of spray material on the phloem of treated trees implies that carbohydrate transport during the next season will not be at adequate levels below the 25-foot level.

Secondary xylem was largely unaffected in treated trees. Resin ducts and ray parenchyma were only rarely affected in the xylem, but tracheids were observed in several regions to lack pits and have distorted secondary wall formation. No unusual resin buildups were observed in resin ducts with the great majority of xylem tissue appearing unaffected. Due to the isolated occurrence of structural damage to this tissue, it is doubtful that the function of this tissue will be seriously affected.

The affect of spray material on the cambium is a key factor in the replacement of affected tissues and the survival of the tree. In general, cambial cells were observed to be generally unaffected with affected cells being isolated to areas where secondary xylem had been adversely affected. With only light damage to the cambium, tissue regenerating

capacities should be present in the 1976 growing season. It is possible, however, that the lack of distinguishable tissues could be related to the nondifferentiation of cells into specific cell types. The presence of newly formed cells in 1975 would indicate functional cambium but a block in the mechanism which specifies cellular differentiation may be speculated. Thus the question arises, that even if the cambium is functional, will it be effective? The effect of possible limited synthate transport on the cambium during the 1976 growing season is yet another factor that could adversely affect the function of the cambium.

Overall, sieve cells appear to be incapable of efficient synthate transport. The most noticable feature observed on damaged cells was the lack of sieve areas and apparent breakdown of cell walls (Figures 3 and 4). In those samples of treated phloem tissue observed, the majority lacked sieve areas with only an occasional "normal" appearing strip of cells being present. If unaffected strip areas are present in sufficient numbers, restrictions of synthate flow may be reduced, but this is expected to occur in only isolated cases. Two-year and older sieve cells in all cases were, as expected, nonfunctional, but they too showed noncharacteristic distortion and breakdown of cell walls. Cell wall damage presents the possibility of treatment materials causing a breakdown of cellulose or other structural components. This hypothesis, or that of nondifferentiation of cell types, could explain the lack of sieve areas and would indicate limited synthate transport from nontreated areas into treated areas of the phloem. Transport restrictions in sieve cells below 25 feet presents the best explanation for the observed exudation. With restricted transport of materials in treated areas, synthates would build up in untreated areas until pressures would cause the material to be exuded from the tree. A chemical analysis of the exudated material should be made to test this hypothesis.

Phloem ray parenchyma was the most adversely affected tissue observed. While other tissues showed some variability in responses to spray material, ray parenchyma was found to be consistently affected. In transverse sections, ray parenchyma, which normally appear as straight bands near the cambium and wavy bands in tissue toward the phellogen, were obscure. Closer examination indicates a collapse of the basic cellular structure (primary cell wall), giving the overall tissue a fine filamentous appearance. Why ray parenchyma would be so adversely affected is not fully understood but could in part be related to structural breakdown. Further study will be required for conclusive evidence of this cause however. Nevertheless, this narrow band of lateral conducting tissue appears to be seriously affected with effective replacement during the 1976 growing season appearing unlikely.

Strand or axial parenchyma, which extends vertically in the inner bark, often appeared to be literally dissolved. In numerous radial sections these cells could not be distinguished from surrounding cell types and were generally observed as one continuous strand of stainable material (Figure 4). Where cells could be distinguished they were plasmolized and large concentrations of stainable material were present when compared to controls. Further down the tree from the 25-foot level, strand parenchyma became less distinguishable due to the absence of the stainable substance that had been present in the upper sections. The stainable substance is speculated to be a synthate material which was not transported more than approximately four feet down the tree from the 25-foot level. In transverse sections, recognizable strand parenchyma cells were observed profusely at the 25-foot level with only a few distinguishable cells at the 21-foot level. Like ray parenchyma, strand parenchyma regeneration at effective levels is doubtful.

While relatively few resin ducts were observed in bark of control trees, they were numerous in treated trees. Also, resin ducts in treated trees were considerably larger than those in nontreated trees. Epithelial cells were few in number and ofter appeared to be lacking. Those present were poorly defined and were coated with a resin-like substance. Definite traumatic resin duct formation was observed (particularly at the 24-25-foot level) indicating a wound reaction by the tree. Resin ducts were observed more frequently in the upper reaches of the sampled area with fewer than expected resin ducts at the 20-21-foot level. Periderm, phellogen, and phelloderm appear adversly affected beyond the possibility of recovery. Typical tissue types in this region were often indistinguishable with frequent, nonstainable, "lump-like" structures and particles of the same material being found throughout this region. What this material is and represents is not known, but it does not appear to be related to suberin or other related compounds. These tissues are not expected to recover and could serve as the first indicator of tree mortality as tissues desiccate and separate from the tree.

CONCLUSIONS

In considering the following conclusions it is important to note that they are based on only three treated lodgepole pine trees that were chosen for their extreme indicativeness of exudate material. Specific information concerning the amounts of spray applied and time of spraying was not available for this study but may be of some significance. Thus, with these factors in mind, the following points may be concluded on the basis of available evidence.

1. Exudation is probably a result of damaged bark tissues and does not result from the limited damage inflicted on active secondary xylem tissues.

2. Buildup and exudation of resin and/or photosynthate materials at the 25-foot level appears to result from the breakdown of primary transporting elements in sprayed phloem areas (i.e., sieve cells and ray paranchyma).

3. Secondary xylem and cambial tissues were affected only lightly. Those affected tracheids lacked pit and secondary wall formation and were generally smaller in size when compared to controls. Cambium appears functional but tissue regenerative capacities could be adversely affected depending on the overall condition and dependence on surrounding tissues.

4. The response of trees to spraying will vary. Mortality is expected (based on these samples) to be very high with most trees beginning to fade during the 1976 season. While high mortality may be expected in 1976, some trees may not fade until the summer of 1977 and others might be expected to show a stripping effect between living and dead tissues. This stripping effect may allow, at least in part, the survival of some trees. The rate of fading and mortality will also be dependent to some degree on environmental conditions.

5. Followup studies and a complete random sampling of treated trees is planned for a better and more thorough understanding of this problem. Nevertheless, #2 fuel oil and related substances are not recommended as a base medium for insecticide spraying on living lodgepole pines in the future. While fuel oil is only indicated as the problem, circumstantial evidence is far too strong to justify the use of this carrier until data to the contrary can be presented.

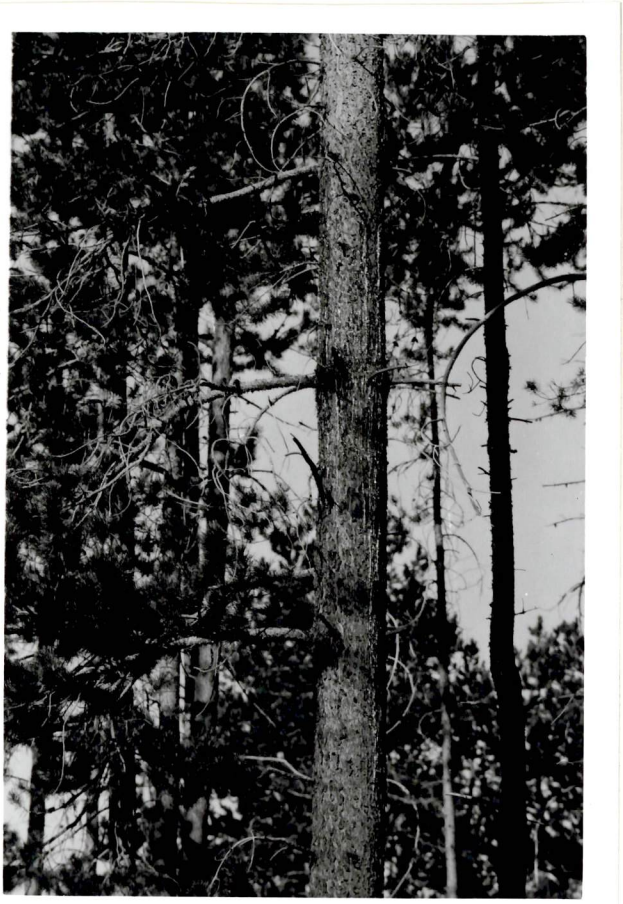

Figure 1. Photo of treatment tree showing phytotoxic reaction to spray material.

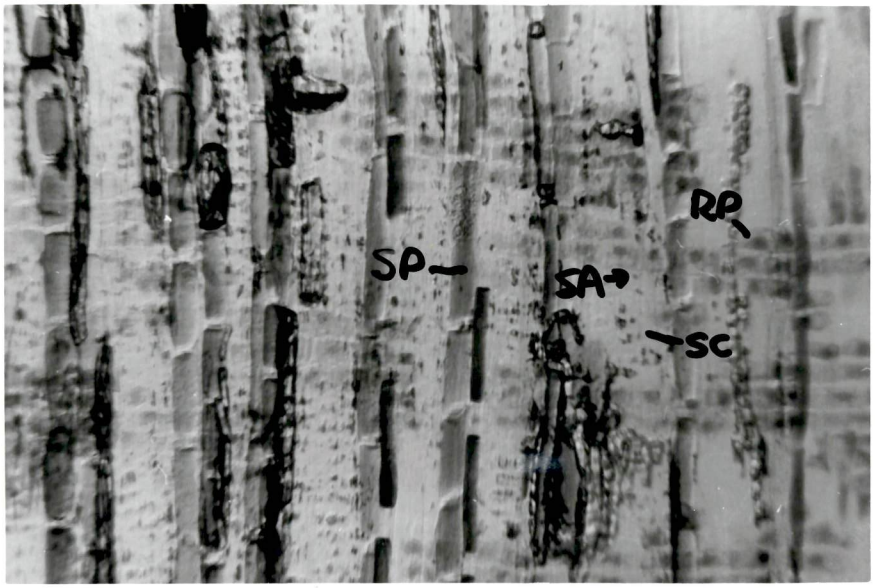

Figure 3. Radial section of nontreated lodgepole pine phloem. RP = Ray Parenchyma, SA = Sieve Area, SC = Sieve Cell, SP = Strand Parenchyma. Cambium is to right of photo.

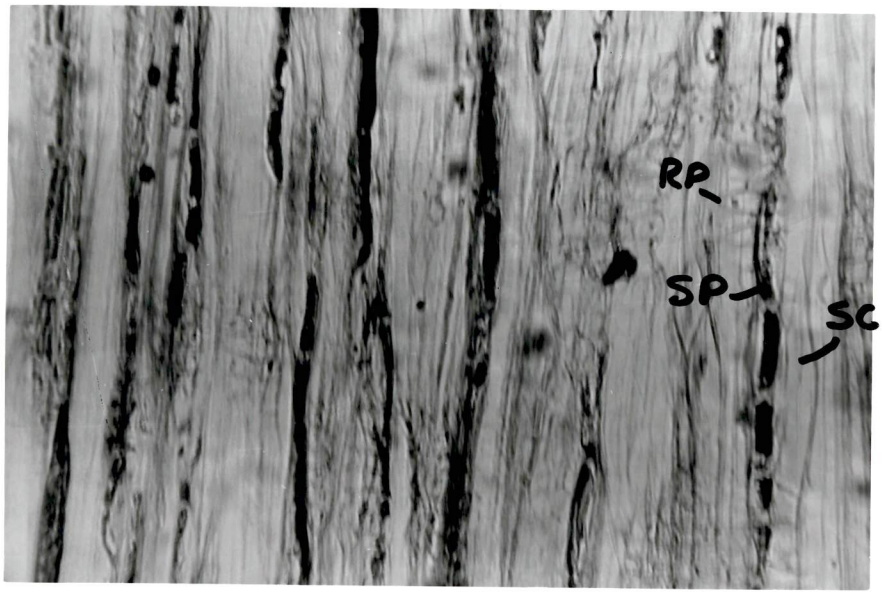

Figure 4. Radial section of treated lodgepole pine phloem at the 23 foot level. RP = Ray Parenchyma, SA = Sieve Area, SC = Sieve Cell, SP = Strand Parenchyma. Cambium is to right of photo.

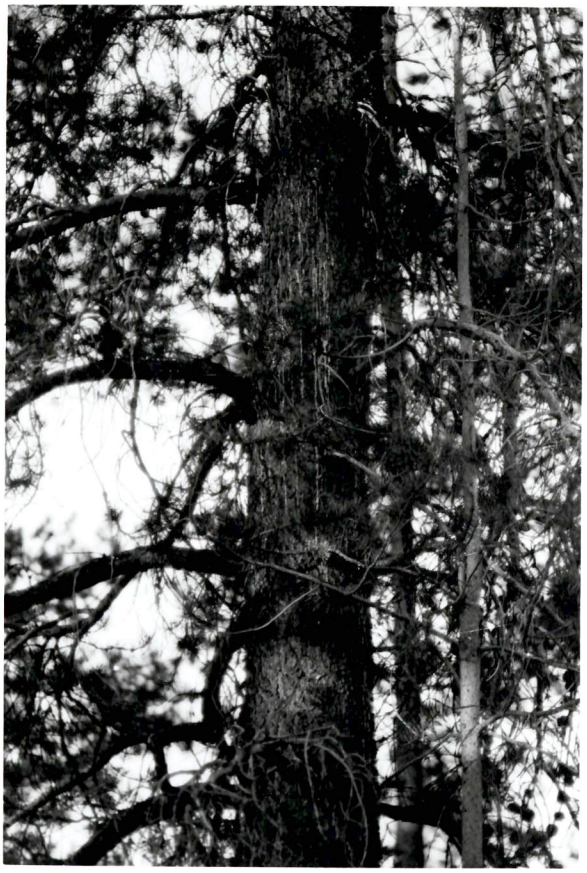

Figure 2. Closeup of treated tree bole in Figure 1. Note exudation of resinous-like substance on tree bole indicating phytotoxic reaction.